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Impact of organic load shock on the dynamic transition of microbial communities during the anaerobic start-up process

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Abstract

This study investigated the shifts of bacterial and archaea diversity suffering the organic load shocks during the start-up. To demonstrate the effects of organic load shock, six reactors was operated in different OLR for 31 days. The community was analyzed using denaturing gradient gel electrophoresis (DGGE) and quantitative PCR. Findings revealed that bacteria diversity was the poorest and archaea quantity was the fewest when suffering OLR shock. MST and MSC were found to the more vulnerable methanogenic archaea groups during organic overloading. Our results explained why gradually increasing OLR is good for favoring the growth of an active biofilm and reactor successful startup quickly during the start-up from the perspective of microbial.

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Keywords: food waste; Organic loading rate; anaerobic digestion; Microbial community

1. Introduction

Food waste (FW), or food loss is the term most often used to describe food materials that are not eaten and subsequently discarded. The disposal of this waste in landfills or even its composting is often regulated due to resulting emission of greenhouse gases, foul odors, and volatile organic compounds. To remedy this, many waste management programs integrate an anaerobic digestion (AD) process, thereby reducing harmful emissions, while simultaneously re-utilizing energy contained in FW. This AD process produces a biogas, which can be used directly as a cooking or heating fuel, to power gas engines, or purified and compressed to natural gas-quality bio-methane thus reducing our dependence on fossil fuels energy sources.

During Anaerobic fermentation, generally, following an initial inoculation period, the organic loading rate (OLR) is increased progressively and carefully monitored to avoid overloading that would otherwise be detrimental to establishment of the microbial biofilm [1]. Although substrate OLR instability during start-up period and its negative effect on these microbial populations are well known, there is unfortunately little data describing microbial responses to the perturbation in the food waste anaerobic

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digestion start-up process. Therefore, our objective in this study was to better understand bacterial and methanogenic population dynamics in an AD system in which OLR fluctuated at the start-up phase.To identify changes in the microbial profiles, we utilized a denaturing gradient gel electrophoresis (DGGE). The responses of archaea communities to the OLR shock were investigated using quantitative real-time polymerase chain reaction (Q-PCR).

2. Methods

The food waste was utlized in this study was obtained from a biogas facility in Lund, Sweden, and consisted of a mixture of grains, vegetables, and meats homogenized using a waring blender to 0.5 mm and stored at –4℃ until used. The total solids (TS) ranged from 8.2 to 12.1% (w/w) and volatile solids (VS) were approximately 80% of TS, and required no pre-treatment prior to use in this study. The inoculum use in this study was also collected from a full-scale mesophilic anaerobic digester in this same Swedish facility. The inoculum TS was 2.2% (w/w) and VS was 60% of TS.

Our experiment consisted of six jacketed (A1, A2, B1, B2, C1, C2), 2 L single-stage glass CSTRs with a working volume of 1.64 L. Reactor A, B and C were represented in the three treatments, and data averaged from two reactors. The FW VS/ inoculum sludge VS was 1/2. The glasses were operated at 60 rpm at 1-minute intervals and the temperature maintained at 37℃ via a thermostatic water bath. Reactors was fed at a different OLR (Table 1)

Table. 1 Organic load changes during the start-up period

Operation stage	Reactor Volum (L)	T (℃)	OLR (kg COD/m^3d)		
			A	B	C
1st stage (1–5d)	2	37	0.99 - 8.25	0.97 - 4.43	1.15 - 6.02
2nd stage (6–8d)	2	37	4.31 - 11.05	4.24 -4.30	4.23 - 11.14
3rd stage (9–12d)	2	37	6.71 - 4.24	6.76 - 4.39	6.75 - 4.37
4th stage (13–31d)	2	37	3.32	3.23	3.24

The methane was analyzed using the Automatic Methane Potential Test System I (Bioprocess Control, Lund Sweden). Biogas composition (methane, carbon dioxide, oxygen, and nitrogen) was determined by a gas chromatograph (Clarus 400 GC, USA). Granular sludge samples were collected from the seed sludge of reactors A, B and C on day 31. Microbial community analyses using DGGE. The PCR primers of 357 F-GC with a GC-clamp, and 517 R were used to amplify the V3 region of the 16S rRNA gene. For methanogenic community Q-PCR analysis, quantitative PCR (Q-PCR) reactions were performed on the ABI 7500 system (Model 7500, Applied Biosystems, USA). The Q-PCR mixture (20μL) was prepared using the 2×TaqMan Universal PCR Master mix (Applied Biosystems, USA): 5 μL of PCR-grade water, 1μL of each primer (final concentration, 10μM), 2μL of the TaqMan probe (final concentration, 1μM), 10 μL of 2×reaction solution, and 1μL of template DNA. The two-step amplification protocol was performed as follows: denaturation for 10 min at 94℃, followed by 40 cycles of 10 s at 94℃, and combined annealing and extension for 30s at 60℃(63℃ was used for only primer set MMB). Four species of the Archaea genera *Methanobacteriales*, *Methanomicrobiales*, *Methanosarcinaceae* and *Methanosaetaceae* were analysed, and the standard strains were provided by the NITE Biological Research Center (NBRC, Chiba, Japan).

3. Results and Discussion

3.1 Performance of the Anaerobic Digestion Reactors

Reactors A and C encountered large fluctuations in feeding (increasing to 8.25 and 6.02 kg COD $m^{-3}d^{-1}$ on the fifth day in the first time OLR shock, and significantly increasing to 11.05 and 11.14 kg COD m-

$^{3}d^{-1}$ in the second time shock), but reactor B feeding was approximately 4.30 COD $m^{-3}d^{-1}$ during 5 to 8 days without OLR changes from table 1. The methane yield from reactor B increased gradually as the OLR was increased, whereas reactors A and C were instable in term of methane yield, pH, methane content, carbon dioxide content and hydrogen content from table 2 obviously due to the effect of OLR shock. Reactors A was the worse than reactor C by reason of strong shock on day 5. From days 12 to31, although the OLR was equal to the A, B and C, operation effect of reactor B was still the best, followed by reactor C seen in the table 2.

Table. 2 Comparison of CSTRs performances on the different OLR

Day	OLR (kg COD/m^3d)			pH			CH4 yield (NL/gVS)			CH_4 (%)			CO_2 (%)			H_2 (ppm)		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
5d	8.25	4.43	6.02	7.02	7.42	7.26	0.07	0.09	0.17	61.25	65.58	62.32	39.23	34.41	37.24	12	9	10
8d	11.05	4.30	11.14	6.85	7.56	7.01	0.09	0.13	0.11	54.44	66.24	59.05	43.96	33.42	39.56	14	8	12
12d	4.24	4.39	4.37	7.23	7.52	7.54	0.32	0.36	0.34	60.85	66.49	63.03	38.96	33.25	35.52	11	8	11
31d	3.32	3.23	3.24	7.45	7.55	7.57	0.53	0.64	0.58	63.86	66.43	64.96	35.73	33.45	34.70	11	9	10

3.2 Bacterial DGGE profiles

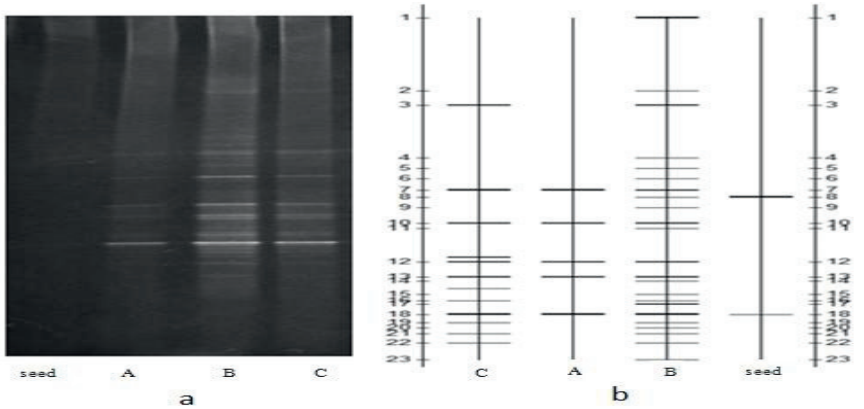

Fig. 1 DGGE profiles of bacterial from seed sludge, reactor A, B and C on day 31

In the fig1, b was reflected the corresponding bands in a figure generated by Quantity One software according to the different rRNA optical density. Compared with seed sludge DGGE profile, it can be seen clearly from figure 1 that reactor B was the most abundant in bacterial diversity with 23 bands thanks to without OLR shock disturbing, only 5 bands were appeared in reactor A DGGE lane due to twice OLR shock on day 5 and 8 and 12 bands were in reactor C DGGE lane. It can be inferred that OLR shock seriously inhibited bacterial diversity in the process of anaerobic fermentation start-up period.

3.5 Quantitative analysis of methanogenicic archaea

Real-time PCR results suggest that the quantitative of MBT, MST, MSC and MMB were completely different when they were suffering different OLR shock. Fig 2 was showed directly from the same seed sludge, four archaea were detected in reactor B and C, with small amount of MST in reactor C， but only MBT and MMB were detected in reactor A on day 31. The MST are known as competitive aceticlastic methanogenics in stable environments at low acetate concentrations [3]. This is confirmed by our finding of a large number of the OLR MST, and lower hydrogen gas concentrations in reactor B. It is well known that MSC is more competitive at high acetate conditions[4]. Therefore, the presence of MSC in post-overload of reactor C may be a response to the accumulation of organic acids resulting from pre-organic overload. MSC was still not appeared in reactor A due to too serious OLR shock. It was showed archaea

quantitative was influenced greatly by OLR shock in the start-up process and leaded to reactor unstable operation.

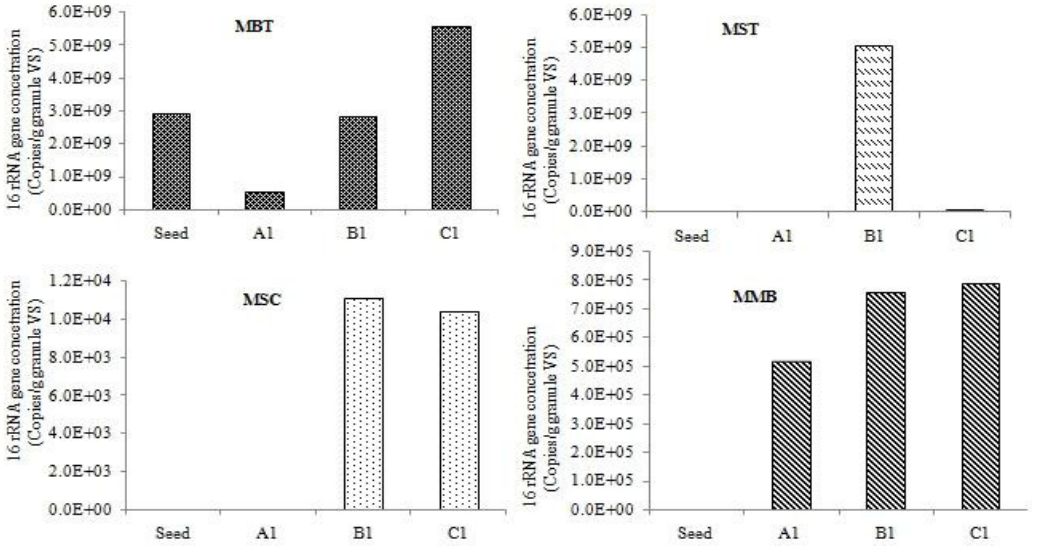

Fig. 2 Quantitative methanogenicic community changes in seed sludge, and reactor A, B and C on day 31

4. Conclusions

In conclusion, our analyses of microbial populations suggested any initial instability directly affects the microbial community. Bacteria diversity was influenced negatively by overload. Methanogenicic archaea quantity was declined suffering OLR shock. MST and MSC were not found in the reactor A during organic overloading. In addition, MSC might be useful as biological indicators for system instability by overload. Our results suggest that gradually increasing OLR is good for reactor successful startup quickly and avoid fluctuation of organic load frequently during running as much as possible for reactor stable efficient operation.

Acknowledgments

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References

[1]. Escudié, R., Cresson, R., Delgenès, J., Bernet, N., 2011. Control of start-up and operation of anaerobic biofilm reactors: an overview of 15 years of research. Water research, 45, 1.

[2]. Apha, A., 1998. WEF (1998) Standard methods for the examination of water and wastewater. American Public Health Association, Washington DC.

[3]. Jetten, M.S., Stams, A.J., Zehnder, A.J., 1990. Acetate threshold values and acetate activating enzymes in methanogenic bacteria. FEMS Microbiology Letters, 73, 339-344.

[4]. Yu, Y., Kim, J., Hwang, S., 2006. Use of real‐time PCR for group‐specific quantification of aceticlastic methanogens in anaerobic processes: Population dynamics and community structures. Biotechnology and bioengineering, 93, 424-433.